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Preventing the recovery of extinguished ethanol tolerance

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ABSTRACT

There is substantial evidence that drug-paired cues become associated with drug effects. From a Pavlovian perspective, these cues act as conditioned stimuli and elicit conditioned compensatory responses that contribute to drug tolerance. Here we report two experiments with rats in which we studied the extinction of the associative tolerance to the ataxic effect of ethanol. Experiment 1 evaluated whether changes in the temporal and physical contexts after extinction training provoke recovery of the extinguished tolerance. The results showed successful extinction, spontaneous recovery and renewal of the extinguished tolerance, but no summation of renewal and spontaneous recovery. Experiment 2 evaluated whether using massive extinction trials and delivering extinction in multiple contexts attenuates the renewal effect. The results showed that both manipulations reduced renewal of the extinguished tolerance to the ataxic effect of ethanol; however, these manipulations used in combination did not appear to be more effective in reducing recovery than each by itself. The present results may help guide further research that evaluates behavioral ploys to prevent the recovery of extinguished responses.

1. Preventing the recovery of extinguished ethanol tolerance

Organisms learn about the relationships between events in their lives through Pavlovian conditioning; with that information they represent their world and act accordingly (Rescorla, 1988). There are several situations in which Pavlovian conditioning is important for the adaptation of organisms to environmental changes (e.g., Domjan, 2005). Of relevance for the present study, Pavlovian conditioning appears to be involved in the development and maintenance of drug tolerance (Siegel et al., 2000), helping organisms to predict and compensate the imminent effects of drugs using the information provided by cues previously paired with them.

From a Pavlovian perspective, the administration of a drug causes a disturbance in the homeostasis of the organism (i.e., unconditioned stimulus, US), to which the body reacts by modifying several physiological parameters (i.e., unconditioned response, UR) that tend to reduce the disturbance and reestablish internal balance (i.e., acute tolerance; Ramsay et al., 1996; Ramsay and Woods, 1997). Interestingly, the effects of drugs become associated with the environmental stimuli (i.e., conditioned stimulus, CS) that were present at the moment of drug intake. Through the pairing of these events, these cues come to elicit a response (i.e., conditioned response, CR) that mimics the compensatory UR elicited in response to the disturbance. This way, the CR anticipates the effects of drugs and helps reduce the disturbance produced by them in the organism (i.e., associative tolerance or chronic tolerance; Ramsay and Woods, 1997). Similarly, when an organism is exposed to stimuli associated with a drug in the absence of the drug itself the compensatory responses occur in reaction to the stimuli, causing what is known as withdrawal symptoms (Ramsay and Woods, 1997; Siegel et al., 2000).

Thus Pavlovian cues are relevant for organisms to anticipate the unconditioned effect of drugs, which in turn has an important role in drug-related cravings and relapses (Childress et al., 1999). Cues associated with drugs also maintain drug-seeking behavior after long periods free of drugs, both in humans and other animals (Gawin and Kleber, 1986; Hellemans et al., 2006; Robinson and Berridge, 2003). More directly related to our study, cues associated with ethanol consumption have also shown to increase appetitive instrumental behaviors without (Krank, 2003) and with demonstrations of associative tolerance (Quezada et al., 2009). For a review on the role of Pavlovian cues in problematic drug use and relapse see Siegel (2001).

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There are several phenomena observed in Pavlovian conditioning that are also present in associative tolerance (e.g., Betancourt et al., 2008b,c; MacRae et al., 1987). One of these is extinction: the reduction of the CR by repeated exposure to CS in the absence of US (Mansfield and Cunningham, 1980; Miguez et al., 2013). Extinction is especially relevant for the treatment of drug abuse disorders (Childress et al., 1988), because through it both drug tolerance and withdrawal symptoms can be reduced, therefore interrupting their role in the maintenance of addictive behavior (Secades-Villa et al., 2007). However, the clinical application of extinction to drug-related disorders has not fulfilled its original expectations (Childress et al., 1988), thus the present research evaluated potential ploys to make extinction-based therapy more effective.

Interestingly, it has been observed that under certain circumstances extinguished responses recover, which indicates that extinction does not erase the original learning but creates a new, possibly inhibitory association that has been shown to be especially context-specific (Bouton, 1993). For example, since the early work of Pavlov it has been reported that extinguished responses recover when a delay is imposed between extinction and testing, a phenomenon known as spontaneous recovery (Pavlov, 1927). In this case, Bouton (1993, 2010) has argued that testing out of the temporal context of extinction is what provokes this type of recovery from extinction. Similarly, recovery from extinction has been reported when testing occurs out of the physical context in which extinction took place, a phenomenon called renewal (Bouton and Bolles, 1979). Additionally, when new CS–US presentations are given after extinction the CR usually develops faster than when such pairings are given to a novel stimulus with US (rapid reacquisition; Ricker and Bouton, 1996); other evidence that suggests the effects of extinction are labile and susceptible to recovery.

These phenomena are fairly robust across a variety of preparations and species (for a review see Bouton, 2002). In the case of associative tolerance, there is evidence for some of the post-extinction response recovery phenomena described above. Brooks et al. (2001) obtained spontaneous recovery of conditioned tolerance to the ataxic effect to ethanol in rats. After successful acquisition and extinction of the tolerance response, subjects were tested with reacquisition CS–US trials after 1, 12, 18, or 24 days from extinction (i.e., retention intervals). Their results showed that tolerance was greater for groups tested 12–24 days following extinction (i.e., it recovered) than for the group tested only 1 day following extinction. This suggests that a relatively long retention interval after extinction spontaneously recovers the extinguished tolerance response. In the case of renewal, the only reported data for associative tolerance to ethanol are those of Betancourt et al. (2008a). Using a similar procedure to the one used by Brooks et al. (2001) and Brooks et al. (2004), they evaluated whether the extinguished tolerance to the ataxic effects of ethanol would recover when acquisition, extinction, and testing occurred in three different contexts. Even though Betancourt et al. found high levels of tolerance to the ataxic effects of ethanol in the context of testing (potential recovery of the extinguished tolerance by testing out of the extinction context), their results should be taken with caution, given that their experiment lacked the critical ABB control group needed to prove this type of renewal (i.e., ABB < ABC).

Various theoretical models have explained both spontaneous recovery and renewal. For example, Bouton's model (1993) proposes that extinction produces an inhibitory memory that interferes with the expression of the acquisition memory. In one version of the model, the extinction memory becomes context-dependent because the CS becomes ambiguous (i.e., it has a history of positive and negative correlation with the US); the association formed during extinction is learned as an exception that can be predicted by contextual cues.

Miller and Laborda (2011) and Laborda and Miller, (2012) extended the ideas of Bouton (1993). For their model, in a given test trial subjects compare the reactivated memory from acquisition and the reactivated memory from extinction. Reactivation of the memories occurs not only due to the strength of the associations learned (i.e., the CS–US memory formed during acquisition and the CS–noUS memory formed during extinction), but also due to the cues from acquisition and/or extinction present at the moment of testing. Therefore, a low response in the test is due to the reactivated memory of extinction being stronger than the reactivated memory of acquisition and vice versa.

Coherently with the previous models, there is evidence supporting the idea that what is learned during extinction is an association with inhibitory characteristics that is easier to express in the spatiotemporal context where extinction occurred than out of that context (Bouton, 1993; Laborda and Miller, 2012). A logical consequence of this is that the differences between the extinction and testing contexts may correlate with the degree of the response recovery found during testing.

In these models summation of the effects of renewal and spontaneous recovery should be expected; changing the physical context and adding a delay from extinction to testing should provoke a greater response recovery than each manipulation by itself. This idea has recently been evaluated by Laborda and Miller (2013) in a fear-conditioned experiment with rats, by Rosas and Bouton (1998) in a taste-aversion preparation with rats, and by Rosas et al. (2001) using a causal judgments paradigm with humans. All reports showed a summative effect of renewal and spontaneous recovery. However, in the case of associative tolerance, the additive effect of spontaneous recovery and renewal has not been explored. Experiment 1 of this report addressed this possibility.

After parameters to evoke high levels of recovery of the extinguished tolerance to the ataxic effect of ethanol were found in Experiment 1, in Experiment 2 we evaluated potential means to disrupt recovery. Recovery from extinction has been established as a model of relapse after exposure treatment for drug-related disorders (Brooks et al., 2004), thus identifying ways to prevent recovery of extinguished tolerance could shed light concerning schemes to prevent lapse and relapse after cue-exposure treatment for alcohol addiction. In Experiment 2 we assessed the effects of giving massive extinction (i.e., a large number of extinction trials) and carrying out extinction in multiple contexts in the recovery of extinguished tolerance. These techniques have previously shown to reduce the recovery of an extinguished response in other preparations (e.g., Chelonis et al., 1999; Denniston et al., 2003; Glautier and Elgueta, 2009; Gunther et al., 1998; Laborda and Miller, 2013), and in other types of associative interference (e.g., Miguez et al., 2014).

2. Experiment 1

Using a similar preparation to that used by Siegel and Larson (1996) to train and assess associative tolerance to the ataxic effect of ethanol, in a 2 (Retention: Short vs. Long) by 2 (Context: Same vs. Different; relative to extinction context) factorial design we manipulated the time elapsed since extinction and the context in which testing was carried out. As shown in Table 1, all subjects had acquisition training where an auditory cue was presented in compound with the effect of an ethanol injection in Context A, followed by an extinction phase where the auditory cue was presented alone in Context B. In the subsequent test, Condition Same was tested in Context B, while Condition Different was tested in a third context, C. In turn, Condition Short (i.e., Groups Short–Same and Short–Different) was tested a day following extinction, whereas Condition Long (i.e., Groups Long–Same and Long–Different) was tested 15 days following extinction. If the effects of both

Table 1
Design of Experiment 1.

Group	Acquisition	Extinction	Delay	Test	Expected
Short–Same	20 $X+_A$	12 $X-_B/_C$	1 day	$X+_B$	cr
Short–Different	20 $X+_A$	12 $X-_B/_C$	1 day	$X+_C$	Cr
Long–Same	20 $X+_A$	12 $X-_B/_C$	15 days	$X+_B$	Cr
Long–Different	20 $X+_A$	12 $X-_B/_C$	15 days	$X+_C$	CR

Note: X = tone. + = effect of ethanol in the organism. - = no US. Subscripts A, B, and C = different contexts. cr, Cr, and CR = different levels of conditioned response expected. Numbers before X = total number of trials.

spontaneous recovery and renewal are found, then Group Short–Same would be expected to present the smallest amount of recovery. Also, if the effects of a physical and a temporal context change are additive, Group Long–Different is expected to have a recovery greater than Groups Short–Different and Long–Same.

2.1. Method

2.1.1. Subjects

Forty-eight experimentally naïve male Sprague-Dawley rats bred at the Facultad de Ciencias Biomedicas of the Universidad Católica de Chile were separated into 4 groups (ns = 12); they weighed an average of 203 g at the beginning of the experiment (ranging from 153 to 215 g). All rats were housed individually at the Laboratorio de Psicología Experimental: Prof. Ronald Betancourt Mainhard of the Departamento de Psicología of the Universidad de Chile, in a room maintained on a 12:12 h light/dark cycle, with food and water available ad libitum throughout the experiment under controlled temperature (22 ± 2 °C). The subjects were weighed daily to adjust the administered doses of ethanol. The ethics committee of the Facultad de Ciencias of the Universidad de Chile approved all procedures used in the experiments reported here.

2.1.2. Apparatus

Four tilting planes, located in pairs in two homologous rooms were used. Each tilting plane consisted of a polycarbonate alley (i.e., a box without ceiling) measuring 60 cm long × 20 cm wide × 30 cm high. One 20 cm side was connected at its upper end by a cord of 1 mm to a pulley and a motor mounted in an iron tower so that when the motor was activated, it pulled the rope and tilted the box 4° per second. On the other side, a protractor located parallel to one of the long walls of the box and perpendicular to its base was used to record the inclination of the box from 0 to 90°. A tilting plane allows evaluating motor coordination by measuring slips. We behaviorally defined a "slip" as the moment in which the rat lost the support offered by the contraction or extension of at least two of its legs, and started displacing downwards in the plane. When a slip occurred we stopped the motor of the tilting the plane, and the inclination angle of the plane was recorded (slip angle).

The US was the ataxic effect of the intraperitoneal injection of ethanol (95%), diluted with physiological saline to a 20% solution. The administered dose was 1.85 g/kg ethanol (Siegel and Larson, 1996). The CS was a 4-min tone (1 kHz) of 68 db (c-scale) generated with an NCH Tone Generator® and the free open-source audio editor software Audacity® (http://audacity.sourceforge.net/), and delivered using two Genius® speakers (100 W).

Three contexts were used, created with different wall covers, odors and lighting of the experimental rooms. One context had transparent box walls and was illuminated using a fluorescent tube located on the ceiling of the experimental room; this context served for pre-exposure and acquisition training (Context A) for all groups. This first context was cleaned with a 3:7 ethanol–water solution. The second context had an orange odor (from a wooden cube with two drops of Gourmet® orange flavoring located approximately 10 cm away from the box), black walls and opaque incandescent light from a 60 W light bulb located under the iron tower of each tilting plane. Finally, the third context had a vanilla odor (achieved by the same means as the orange odor), white walls and a red light from a 12 W light bulb located under the iron tower of each tilting plane. The second and third contexts were cleaned with water and were counterbalanced to serve as the extinction context (B) and the recovery context (C).

2.1.3. Procedure

The experiment was planned so that all subjects were tested the same day. Therefore, Condition Long began training 15 days before Condition Short.

2.1.3.1. Acclimation. All rats were handled daily for 7 days. This handling was the same needed for the injection procedure, which consisted of wrapping the rat with a lace curtain cloth to immobilize the legs, leaving the abdomen exposed and holding them in the palm of the hand of the experimenter for about 30 s.

2.1.3.2. Pre-exposure. Pre-exposure to the contextual and injection cues occurred on Days 1–10 for Condition Long, and on Days 15–24 for Condition Short. All rats received an intraperitoneal injection of saline solution 3 times per day, in sessions separated by 4 h in Context A; emulating the treatment that they would receive in the acquisition phase. After the injection the subjects were immediately placed in the tilting plane for 4 min, measuring their ataxic response at 2 and 4 min after the saline injection. Slip angles were measured by the experimenter by placing the rat at the unhinged end of the tilting plane and elevating it until the rat slipped to the hinged end. This phase was included to reduce the possible competition between injection cues and the target stimulus. Data from these trials were not analyzed.

2.1.3.3. Acquisition. Acquisition training occurred on days 11–20 for Condition Long, and on days 25–34 for Condition Short. All subjects were injected with the ethanol solution two times each day in sessions separated by 4 h in Context A. For each trial, rats were first placed into the tilting plane to measure two consecutive slip angles that served as a baseline. Then they were injected with the ethanol solution and immediately placed on the tilting plane again for 4 min with simultaneous presentation of the CS. Measures of two slip angles were made at 2 and 4 min after the onset of the CS to evaluate the ataxic response. An impairment score for each trial was calculated by subtracting the average of the baseline measurements to the smaller of the measurements at 2 and 4 min after the CS onset. The two daily impairment scores were averaged to conform blocks per session for data analysis purposes.

2.1.3.4. Extinction. Extinction training occurred on days 21–23 for Condition Long and on days 35–37 for Condition Short. All rats received 12 individual CS presentations in Context B. The CS were presented with intertrial intervals (ITI) of 6 s. Additionally, during these 3 days subjects were also exposed to Context C without presentation of any nominal stimuli to match the total exposure time to Context B and C, thus reducing the possibility that the presentation of a new context could increase the ataxic response in the test in Context C (i.e., external inhibition; Larson and Siegel, 1998). The order of the sessions (i.e., extinction or exposure session first) was counterbalanced across days. Sessions were separated by 4 h.

2.1.3.5. Test. To measure response recovery after extinction, all subjects received 2 daily reacquisition trials on days 38 and 39, following the same procedure used during acquisition training. Rats in Condition Same were tested in the extinction context (i.e., Context B), while rats in Condition Different were tested in Context C.

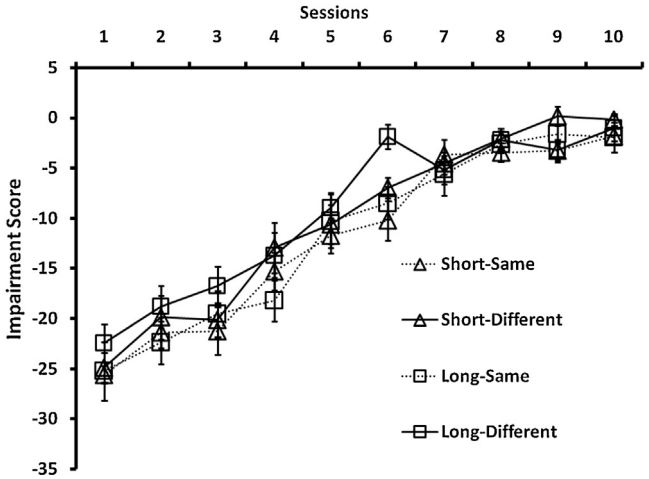

Fig. 1. Mean impairment scores for each acquisition session per group in Experiment 1. See Table 1 for treatments. Scores closer to zero indicate greater conditioned tolerance. The graph shows the acquisition of tolerance to the ataxic effect of ethanol for all groups. Error bars represent standard error.

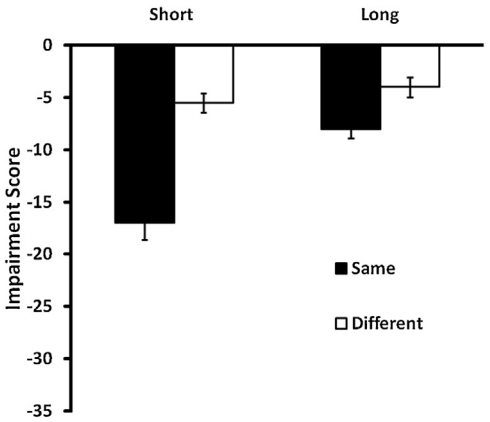

Fig. 2. Reacquisition test. Scores closer to zero indicate greater conditioned tolerance. The graph shows the reacquisition of the tolerance response by groups. Error bars represent standard error.

This time, all impairment scores were averaged for data analysis purposes.

2.1.4. Results

To analyze the impairment scores of acquisition data (Fig. 1), a mixed model analysis of variance (ANOVA) was performed, using Session (10 acquisition sessions) as the within-subject factor and Retention (2: Short and Long) and Context (2: Same and Different) as between-subject factors. The analysis shows that subjects significantly decreased their impairment scores as a function of Sessions, $F_{(9,396)} = 164.03$, $p < 0.01$, MSE = 3551.7, $\eta^2_p = 0.79$, demonstrating that the rats developed associative tolerance to the ataxic effects of ethanol. No other main effects or interactions were found (all ps > 0.05). Of some interest, it can be argued that the decrease in the impairment scores during acquisition training is a result of the subjects adapting their behavior to the testing situation. However, a repeated measures ANOVA applied to the baseline data across acquisition sessions suggests that is not the case, $F_{(9,423)} = 1.98$, $p < 0.05$, MSE = 10.8, $\eta^2_p = 0.04$. A planned comparison between the baseline data from session 1 and session 10 resulted significant, $F_{(1,47)} = 4.05$, $p < 0.05$, supporting the idea that training did not improve responding at the tilting plane, if anything, the performance slightly deteriorated during acquisition training (session 1: $M = 42.94$, SD = 3.98, IC 95% [41.78, 44,10]; session 10: $M = 41.79$, SD = 3.21, IC 95% [40.86, 42.72]).

To assess the effect of the extinction procedure, another mixed model ANOVA was performed using Trial (2; considering the last acquisition trial and the first reacquisition trial) as the within-subject factor and Context (2: Same and Different) and Retention (2: Short and Long) as between-subject factors. An effect of Trial was observed, $F_{(1,44)} = 87.76$, $p < 0.01$, MSE = 1807.0, $\eta^2_p = 0.67$, which indicates that the tolerance to the ataxic effect of ethanol was successfully extinguished with our procedure. No other main effects or interactions were found (all ps > 0.05).

Finally, to assess the additive effect of a temporal and physical context change a factorial ANOVA was performed on the test data, using Context (2: Same and Different) and Retention (2: Short and Long) as between-subject factors (Fig. 2). The analysis showed that there was a significant effect of Context, $F_{(1,44)} = 45.22$, $p < 0.01$, MSE = 721.72, $\eta^2_p = 0.51$, which indicates that imposing a delay between extinction and testing provoked spontaneous recovery; a significant effect of Retention, $F_{(1,44)} = 20.43$, $p < 0.01$, MSE = 326.17, $\eta^2_p = 0.32$, which indicates that testing out of the extinction context induced renewal; and an interaction between these two factors, $F_{(1,44)} = 10.31$, $p < 0.01$, MSE = 164. 56, $\eta^2_p = 0.19$, which suggests that testing out of the extinction context (i.e., renewal) provoked more recovery when a short delay between extinction and testing was imposed than when the delay was long.

Planned comparisons were performed using the error term of the factorial ANOVA to test specific hypotheses and to detect the source of the interaction. Lower impairment scores were observed in Group Short–Different than in Group Short–Same, $F_{(1,44)} = 49.36$, $p < 0.01$, indicating that the extinguished tolerance to the ataxic effect of ethanol was renewed. Also, lower impairment scores were obtained in Group Long–Same relative to Group Short–Same, $F_{(1,44)} = 29.89$, $p < 0.01$, suggesting the presence of spontaneous recovery of the extinguished tolerance. Additionally, Group Long–Different had smaller impairment scores relative to Group Long–Same, $F_{(1,44)} = 6.17$, $p < 0.05$, suggesting that a spatiotemporal context change provoked more recovery of extinguished tolerance than a temporal context change by itself; however, the Short–Different and Long–Different groups did not differ ($p = 0.36$), indicating that with our parameters there was no more recovery of the extinguished tolerance to ethanol after a physical context change from extinction (i.e., renewal) than after a conjoint change in the physical and temporal contexts.

Notice that, in order to measure associative tolerance, we have to evaluate the effect of ethanol during the CS. Measuring the CS alone during extinction training correspond to evaluate an abstinence response (i.e., withdrawal response; the compensatory responses elicited by the CS with no drug effects to counteract) which could not be compared with the data from acquisition. Even if we assessed slip angles during extinction, our instrument to measure ataxia (i.e., tilting plane) is not sensitive enough to evaluate scores higher than those from the baseline.

In summary, a successful acquisition of associative tolerance to the ataxic effects of ethanol and an effective extinction of this tolerance were observed. In addition, recovery of the extinguished response was observed both testing out of the extinction context and after a long delay after extinction; that is, renewal and spontaneous recovery of the associative tolerance to ethanol were observed. However, the effect of imposing a delay between extinction and testing was not appreciable when testing was carried out in a new context. It is likely that, due to the nature of our measurement, a ceiling effect occurred preventing us from observing the additive properties of spontaneous recovery and renewal that have been found in other studies (Laborda and Miller, 2013; Rosas and Bouton, 1998; Rosas et al., 2001). Based on this last result, the fol-

Table 2
Design of Experiment 2.

Group	Acquisition	Extinction	Test	Expected
Single–Few	16 $X+_A$	12 $X-_B$	$X+_E$	CR
Single–Many	16 $X+_A$	60 $X-_B$	$X+_E$	Cr
Multiple–Few	16 $X+_A$	$4X-_B/4X-_C/4X-_D$	$X+_E$	Cr
Multiple–Many	16 $X+_A$	$20X-_B/20X-_C/20X-_D$	$X+_E$	cr

Note: X = tone. + = effect of ethanol in the organism. Subscripts A, B, and C = different contexts. cr, Cr, and CR = different levels of conditioned response expected. Numbers before X = total number of trials.

Table 3
Exposure and extinction cycle in Experiment 2.

Group/Day	1	2	3	4	5	6	7	8	9	10	11	12
Single–Few	E	D	C	2X- B	E	D	C	2X- B	E	D	C	2X- B
Single–Many	E	D	C	10X- B	E	D	C	10X- B	E	D	C	10X- B
Multiple–Few	E	D	C	2X- B	E	D	B	2X- C	E	B	C	2X- D
Multiple–Many	E	D	C	10X- B	E	D	B	10X- C	E	B	C	10X- D

Note:1–3, 5–7, and 9–11 were context exposure days; 4, 8 and 12 were extinction days. X = tone. - = no US. A, B, C, D and E = different contexts. Numbers before X = number of trials on that day.

lowing experiment used a renewal preparation to assess recovery prevention techniques.

3. Experiment 2

In Experiment 2 we evaluated two techniques that have shown to reduce the recovery of an extinguished response in other Pavlovian conditioning procedures: giving massive extinction, and performing extinction in multiple contexts. For this experiment we decided to shorten the acquisition phase from 20 to 16 trials (and proportionally, the pre-exposure phase) because what appeared to be asymptotic levels of tolerance were reached at day 8 of the acquisition phase in the previous experiment. This time we manipulated the number of extinction trials (Few vs. Many) and the number of extinction contexts (Single vs. Multiple), in a 2 by 2-factorial design (see Table 2). As both techniques have been reported to reduce the amount of fear recovery, we expected to observe less recovery of the tolerance response when many instead of few trials were given, and also when multiple contexts instead of a single one were used during extinction. Also, we expected these effects to be additive, as found by Laborda and Miller (2013).

3.1. Method

3.1.1. Subjects

Forty-eight experimentally naïve male Sprague-Dawley rats bred at the Faculty of Chemistry and Pharmaceutical Sciences of the Universidad de Chile were separated into 4 groups (ns = 12); they weighed an average of 232 g at the beginning of the experiment (ranging from 186 to 304 g). All rats were housed and maintained as in Experiment 1.

3.1.2. Apparatus

The same instruments and stimuli of Experiment 1 were used. This time, we added two new features to the context pool. The fourth context had coconut odor (Gourmet® coconut flavoring), walls designed with black and white vertical lines of 24 mm width and a 40 W incandescent light, flashing at a 1 s intermittency. The fifth context had a mint odor (Gourmet® mint flavoring), walls with a checkered design (squares of 5 cm sides) and a 40 W incandescent light, flashing at a 0.5 s intermittency. As in Experiment 1, the first context was used for the pre-exposure and acquisition phases (Context A), while the other four contexts were counterbalanced to be the extinction, exposure, and testing contexts.

3.1.3. Procedure

The handling, pre-exposure and acquisition phases were identical to those of Experiment 1, the only difference being the number of trials used for pre-exposure and acquisition (pre-exposure trials were reduced from 30 to 24 trials and acquisition trials from 20 to 16 trials). For all subjects the pre-exposure phase took place on days 1–8, while the acquisition phase occurred on days 9–16.

3.1.3.1. Extinction. For 24 days all rats received extinction of the CS and equal exposure to Contexts B–E. Extinction sessions lasted 41 min and occurred every 4 days for all groups (Days 4, 8, 12, 16, 20 and 24; see Table 3). Exposure sessions had the same duration and took place on Days 1–3, 5–7, 9–11, 13–15, 17–19 and 21–23 for all subjects. Regarding the number of contexts used in extinction, Condition Single received all extinction trials in Context B, while Condition Multiple received extinction trials equally divided among Contexts B–D. Regarding the number of extinction trials, Condition Few received 12 extinction trials in total, while Condition Many received 60 extinction trials in total, both equally divided among the six extinction sessions. All extinction trials had 6 s ITI. To keep contextual exposure and ITI constant along groups, Condition Few had their first extinction trial in each session presented after 32 min and 48 s, and then continued with 6 s ITI, while Condition Many used 6 s ITI from the beginning of each extinction session.

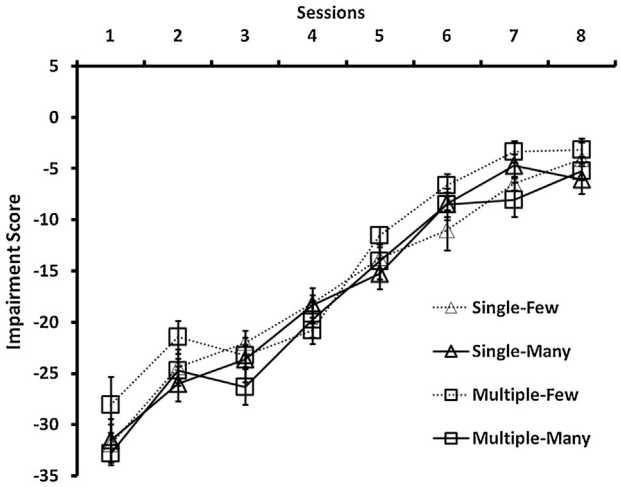

Fig. 3. Mean impairment scores for each acquisition session per group in Experiment 2. See Table 2 for treatments. Scores closer to zero indicate greater conditioned tolerance. The graph shows the acquisition of tolerance to the ataxic effect of ethanol for all groups. Error bars represent standard error.

3.1.3.2. Test. The test was exactly as in Experiment 1, but this time all subjects were tested in a neutral but familiar context (i.e., Context E).

3.2. Results

To analyze the acquisition data (Fig. 3) a mixed model ANOVA was performed, using Session (8 acquisition sessions) as the within-subject factor and Contexts (2: Single and Multiple) and Trials (2: Few and Many) as between-subject factors. The analysis shows that subjects significantly decreased their impairment scores as a function of Session, $F_{(7,308)} = 197.46$, $p < 0.01$, MSE = 45.32., $\eta^2_p = 0.82$, showing that our rats developed associative tolerance to the ataxic effects of ethanol. No other main effects or interactions were found (all ps > 0.05). Of some interest, it can be argued that the decrease in the impairment scores during acquisition training is a result of the subjects adapting their behavior to the testing situation. However,

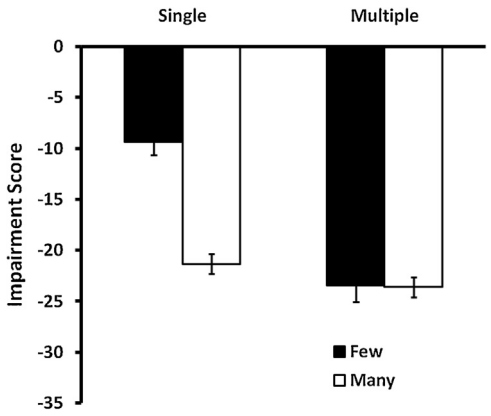

Fig. 4. Reacquisition test. Scores closer to zero indicate greater conditioned tolerance. The graph shows the reacquisition of the tolerance response by groups. Error bars represent standard error.

a repeated measures ANOVA applied to the baseline data across acquisition sessions suggests that is not the case, $F_{(7,329)} = 10.38$, $p < 0.05$, MSE = 68.5, $\eta^2_p = 0.18$. A planned comparison between the baseline data from session 1 and session 8 failed to reach significance ($p = 0.54$), supporting the idea that training did not improve responding at the tilting plane (session 1: $M = 45.17$, SD = 4.07, IC 95% [43.99, 46.35]; session 8: $M = 45.52$, SD = 2.90, IC 95% [44.67, 46.36]).

To assess the additive effects of massive extinction and extinction in multiple contexts a factorial ANOVA was performed on the test results, using Contexts (2: Single and Multiple) and Trials (2: Few and Many) as between-subject factors (Fig. 4). The analyses showed a significant effect of Context, $F_{(1,44)} = 42.55$, $p < 0.01$ MSE = 806.47, $\eta^2_p = 0.49$, indicating that the use of multiple extinction contexts was effective in reducing the recovery of the ataxic response; a significant effect of Trials, $F_{(1,44)} = 23.35$, $p < 0.01$ MSE = 442.56, $\eta^2_p = 0.35$, showed that the use of massive extinction was successful in reducing the recovery of the ataxic effect of the tolerance; and an interaction between these two factors, $F_{(1,44)} = 22.24$, $p < 0.01$ MSE = 421.56, $\eta^2_p = 0.34$, indicating that adding a second technique did not reduce renewal more than each technique by itself.

Planned comparisons were performed using the error term of the factorial ANOVA to test specific hypotheses and to detect the source of the interaction. Lower impairment scores were observed in Group Single–Few than in Group Single–Many, $F_{(1,44)} = 45.59$, $p < 0.01$, showing that increasing the extinction trials reduced the renewal of extinguished tolerance to the ataxic response. Also, lower impairment scores were obtained in Group Single–Few relative to Group Multiple–Few, $F_{(1,44)} = 63.17$, $p < 0.01$, suggesting that increasing the number of extinction contexts reduced the recovery of the extinguished response. However, the Multiple–Few and Multiple–Many groups and the Single–Many and Multiple–Many groups did not differ (both $p \geq 0.21$), indicating that with our parameters the use of each technique by itself was no different from using many trials of extinction conducted in multiple contexts.

Experiment 2 replicated the renewal of the extinguished tolerance to the ataxic effects of ethanol observed in Experiment 1. Furthermore, the use of massive extinction trials and multiple contexts in extinction were effective in reducing response recovery. However, these techniques used in combination did not appear to be more effective than each technique by itself. Probably, using any of these techniques by itself seems to prevent recovery effectively, not allowing any benefit of adding a second technique to be observed (i.e., floor effect).

4. General discussion

The aims of these experiments were: (a) to evaluate whether temporal and physical contextual changes have additive effects, increasing the recovery of extinguished tolerance to the ataxic effect of ethanol more than either contextual change alone (Experiment 1), as has been found in other studies (Laborda and Miller, 2013; Rosas and Bouton, 1998; Rosas et al., 2001); (b) and to evaluate the individual and combined effects of using massive extinction trials and performing extinction training in multiple contexts in the renewal of extinguished tolerance to the ataxic effect of ethanol (Experiment 2).

In Experiment 1 we replicated the results of Brooks et al. (2004) and Brooks et al. (2001), finding spontaneous recovery of the extinguished tolerance to the ataxic effect of ethanol. Also, overcoming the methodological issues of Betancourt et al. (2008a), we presented evidence of the renewal of extinguished ethanol tolerance. Of interest, we found that subjects who received a spatiotemporal context change (i.e., renewal plus spontaneous recovery) had significantly more recovery of the extinguished response than subjects that received only a temporal context change (i.e., spontaneous recovery). However, we did not find a difference between the recovery caused by a spatiotemporal context change and the physical contextual change by itself (i.e., renewal). It seems that renewal by itself was strong enough, and adding a temporal context change did not further increase the recovery (i.e., ceiling effect). As a recovery model of relapse in clinical settings, a high level of recovery could allow researchers to evaluate the additive effects of behavioral manipulations to prevent recovery, and thus inform clinicians concerning potential ploys to enhance cue-exposure therapies for drug-related problems and reduce relapse. Also, using combined temporal and physical context changes to evaluate recovery seems a little more natural, as relapse in real life often occurs in new situations and after a time from exposure to treatment.

In Experiment 2, renewal of the extinguished tolerance to the ataxic effect of ethanol was replicated. Interestingly, both massive extinction trials and extinction in multiple contexts reduced renewal of ethanol tolerance. As in fear-conditioning preparations (Denniston et al., 2003; Laborda and Miller, 2013), using an extensive number of extinction trials attenuated renewal in an ethanol tolerance preparation. With respect to using multiple extinction contexts, unlike Betancourt et al. (2008a), we did find an attenuation of the renewal of extinguished tolerance to the ataxic effect of ethanol, a difference likely explained by differences in the parameters used in each study. Betancourt et al. used 21 acquisition trials and 9 extinction trials whereas we used 20 and 16 acquisition trials (Experiments 1 and 2, respectively) and 12 extinction trials. Together, more acquisition and less extinction training could provoke poorer extinction effects in Betancourt et al. compared to our present results, which suggests it is likely that our parameters could yield more extinction and therefore provide an increased range for response recovery (i.e., more sensitivity to recovery). Our results increase the positive reports of using extinction in multiple contexts to prevent recovery in different preparations (e.g., fear conditioning in rats; Gunther et al., 1998; Laborda and Miller, 2013; taste aversion in rats, Chelonis et al., 1999; and startle blink response in humans, Bandarian Balooch et al., 2012; but see Bouton et al., 2006; Neumann et al., 2007). Finally, using massive extinction in multiple contexts did not reduce recovery more than each technique by itself, as was reported in a fear conditioning preparation (Laborda and Miller, 2013). This inconsistency may be due to a floor effect produced by each of the techniques, making it impossible to observe more recovery prevention.

To be noted, the results of Experiment 1 and 2 cannot be explained by the subjects adapting their behavior to the test situation, as the analysis of the baseline data from acquisition train-

ing showed. If anything, baseline responding across acquisition sessions slightly deteriorated, it did not improve as expected if responding was a function of training in the tilting plane.

On the whole, the two experiments provided relevant evidence in the associative tolerance of ethanol paradigm at both theoretical and applied levels. Various theoretical models have explained both spontaneous recovery and renewal. In this report we analyzed the results from the perspective of some cognitive theories. For example, Bouton's (1993) model proposes that extinction produces an inhibitory memory that interferes with the expression of the acquisition memory. In one version of the model, the extinction memory becomes context-dependent because the CS becomes ambiguous (i.e., it has a history of positive and negative correlation with the US); in this case, the association formed during extinction is learned as an exception that can be predicted by contextual cues.

Miller and Laborda (2011) and Laborda and Miller (2012) extended the ideas of Bouton (1993). For their model, in a given test trial subjects compare the reactivated memory from acquisition and the reactivated memory from extinction. Reactivation of the memories occurs not only due to the strength of the associations learned (i.e., the CS–US memory formed during acquisition and the CS–noUS memory formed during extinction), but also due to the cues from acquisition and/or extinction present at the moment of testing. Therefore, a low response in the test is due to the reactivated memory of extinction being stronger than the reactivated memory of acquisition and vice versa.

Consistent with the previous models described above, there is also evidence supporting the hypothesis that what is learned during extinction is an inhibitory relation that is readily evoked in the spatiotemporal context in which training occurred rather than outside that context (Bouton, 1993; Laborda and Miller, 2012). A logical consequence of this is that the differences between the extinction and testing contexts may correlate with the degree of the response recovery found during testing.

On a theoretical level, the results of Experiment 1 can be partially explained by both Bouton's (1993) and Miller and Laborda's (2011) extinction models, as both proposed that changes in the temporal and physical context of extinction would induce recovery (both predict renewal and spontaneous recovery). However, both models predict that the differences in the spatiotemporal context of testing relative to the extinction context should correlate with more recovery, which was not found in our study, most likely due to a ceiling effect.

The results of Experiment 2 can also be partially explained by Bouton's (1993) extinction model. For Bouton, reduced renewal should be expected with extinction in multiple contexts (as was found in the present study), because this manipulation should increase the generalization of extinction learning to other contexts. However, Bouton's model cannot explain why extensive extinction reduces renewal (as was found in the present study). His model actually predicts more contextual specificity of extinction (i.e., more renewal) with more extinction trials, and not less specificity as was found in our study. Of interest, Miller and Laborda's (2011) model of extinction can accommodate both major results of Experiment 2. For them, extinction in multiple contexts should increase the strength of the reactivated memory of extinction when tested by increasing generalization of the extinction learning, thereby reducing response recovery. Also, for Miller and Laborda, extensive extinction training should increase the strength of the reactivated memory of extinction when tested by increasing the CS–noUS association, thereby reducing response recovery. However, this model predicts (contrary to Bouton's model) that the effects of extinction in multiple contexts and massive extinction should sum, further reducing recovery, which was not found in the present study.

At an applied level, these findings are particularly relevant for clinicians, considering that cue-exposure treatments (CET) for drug-related disorders have not been very effective. The results presented here could help improve this type of therapy by implementing in the clinical environment techniques that have proved to reduce the recovery of extinguished responses in basic research experiments (Laborda et al., 2011; Vervliet et al., 2013). However, the successful use of these techniques in clinical research has not been easy, and mixed results have been found. For example, Mackillop and Lisman (2008) used a laboratory analogue of CET to evaluate the occurrence of the renewal of extinguished reactivity to alcohol cues in heavy drinkers. They also assessed whether the use of multiple extinction contexts could attenuate renewal in their preparation. Their results suggested that a context shift did not provoke a renewal effect and that the use of multiple contexts during extinction was ineffective in improving the extinction learning. It is necessary to evaluate the critical differences between basic and applied research to find out why the techniques that have proven effective in reducing recovery of the extinguished response in rats fail to reduce the relapse in humans.

Finally, these data may help guide further research concerning other techniques that have yielded positive results in reducing recovery of the extinguished behavior in other studies, and to evaluate whether they are able to mitigate the recovery of extinguished ethanol tolerance.

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